www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2016; 5(7): 41-46

www.thepharmajournal.com
Received: 08-05-2016
Accepted: 09-06-2016

Ivanna Milian
Department of Pharmacognosy with Medical Botany, I Horbachevsky Ternopil State Medical University Ternopil, Ruska 36, 46001, Ukraine

Svitlana Marchyshyn
Department of Pharmacognosy with Medical Botany, I Horbachevsky Ternopil State Medical University Ternopil, Ruska 36, 46001, Ukraine

Solomiia Kozachok
Department of Pharmacognosy with Medical Botany, I Horbachevsky Ternopil State Medical University Ternopil, Ruska 36, 46001, Ukraine

Nazar Yavorivskiy
Student of the 3-rd Course of Medical Faculty, I Horbachevsky Ternopil State Medical University Ternopil, Ruska 36, 46001, Ukraine

Correspondence
Ivanna Milian
Department of Pharmacognosy with Medical Botany, I Horbachevsky Ternopil State Medical University Ternopil, Ruska 36, 46001, Ukraine

Investigation of phenolic compounds of the herbs of *Veronica* Genus

Ivanna Milian, Svitlana Marchyshyn, Solomiia Kozachok and Nazar Yavorivskiy

Abstract
It was determined phenolic compounds of the herb of *Veronica prostrata* L., *Veronica chamaedrys* L., *Veronica officinalis* L. by the method of high performance liquid chromatography (HPLC). Established quality composition and quantity content of flavonoids, hydroxycinnamic acids, coumarins and hydrolysable tannins. In the herb of *Veronica prostrata* L. it was revealed 5 hydroxycinnamic acids, the most abundant was rosmarinic acids – 0,41%, 3 flavonoids, the highest content was for apigenin and its glycosides – 0,34%, coumarin – 0,07%, 7 hydrolysable tannins, the highest content related to catechin – 1,45%; *Veronica chamaedrys* L. – 5 hydroxycinnamic acids (rosmarinic acid 1,31%), 5 flavonoids (rutin 0,26%), 3 coumarins (umbelliferone 0,17%), 5 hydrolysable tannins (epicatechin 0,06% and ellagic acid 0,06%); *Veronica officinalis* L. – 5 hydroxycinnamic acids (rosmarinic acid 1,41%), 2 flavonoids (isoquercetin 0,28%), 2 coumarins (umbelliferone 0,03%), 7 hydrolysable tannins (epigallocatechin 0,44%).

Keywords: *flavonoids, hydroxicinnamic acids, coumarins, tannins, HPLC, Veronica prostrata* L., *Veronica chamaedrys* L., *Veronica officinalis* L.

1. Introduction
The herbs of *Veronica* genus are an abundance source of phenolic compounds (simple phenols, hydroxycinnamic aids – HCA, coumarins – C., tannins – T, flavonoids – Fl), they are applied for the treatment of heart diseases, diabetes, chlamydial infections, reduce the rate of mutagenesis in cells; exhibit antitumor, anti-inflammatory, anti-oxidant, wound-healing activities [7, 8].
The content of phenolic compounds in the herbs of *Veronica prostrata* L., *Veronica chamaedrys* L., *Veronica officinalis* L. are limited studied, thus the aim of our studies was to determine the quality composition and quantity content of phenolic compounds in the studied species of *Veronica* genus by HPLC.

2. Materials and Methods
2.1 The research objects: the subjected plant materials – *Veronica prostrata* L. and *Veronica chamaedrys* L. were harvested from wild plots in Zalischyky, Ternopil region (the Western part of Ukraine) during flowering stage, in 2013 and 2014 respectively. *Veronica officinalis* L. was collected on the outskirts of the city Galych, Ivano-Frankivsk region (the Western part of Ukraine), during flowering stage in 2014. The collected specimens were identified by prof. S.M. Marchyshyn, Department of Pharmacognosy with Medical botany, I. Horbachevsy Ternopil State Medical University.
Chromatographic separation was performed by chromatograph (Agilent 1200 3 D LC System Technologies, USA) with four-channel vacuum degasser G1354 A, autosampler G1329A, autosampler thermostat G133O B, coloumn thermostat G1316A, diode array detector (G1315C) in complex with PC software Agilent ChemStation (G2215 BA), on the column Discovery C_{18}, 250 x 4,6 mm (Supelco, № 505129) with the precolomn of 20 mm with a grain size of 5 µm at the column thermostat temperature 25 °C. Injection of the samples was carried out by autosampler, volume of samples was 5-20 µm, flow rate – 0.7 ml/min [6], 0,8 мл/хв. and 0.5 ml/min [5].
For the separation of phenolic compounds it was applied the following parameters of the chromatographic analysis: gradient elution, mobile phase – bidistilled water acidulated with 0,005 N phosphoric acid solution ("A") and acetonitrile ("B") – analysis of HCA, C and Fl; 0,1% trifluoroacetic acid solution, 5% acetonitrile solution (A) and acetonitrile 0,1% trifluoroacetic acid solution (B) – analysis of T. Scan time was 0,6 seconds, the detection

range – 190-400 nm, the wavelength of ultraviolet spectra detection – 320 (ferulic, *p*-coumaric acids) and 330 nm (HCA, C) and 280 and 255 nm (T) and 340 nm and 255 nm (Fl). Total analysis time was 40 and 50 minutes [1, 3, 5, 6].

Table 1: Parameters of gradient elution

Flavonoids							
Time, min	0	30	33	38	40	41	49-60
Eluant B, %	12	25	25	30	40	80	12
Tannins							
Time, min	0	8	10	15	20	25	28	29-40
Eluant B, %	100	12	12	25	25	75	75	100
Hydroxycinnamic acids							
Time, min	0	8	15	30	40	41	43-50
Eluant B, %	5	8	10	20	40	75	5

2.2 Sample preparation. grinded sample material, carefully selected about 1,00-2,00 g (accurately mass) and placed into a flat-bottomed flask on 100 cm^3, extracted with 50 cm^3 of 60% methanol solution and the flask joined to the reflux condenser for 30 minutes, heated on a magnetic mixer at 70 ^{0}C. After the sample was treated with ultrasound for 10 minutes at a frequency of 45 kHz at 70 °C. The obtained extracts was cooled and quantitatively transferred into the volumetric flask on 100 cm^3, enhanced the volume up to mark by 60% methanol solution [4]. The hydrolyzable tannins of the studied objects extracted with 50 cm^3 of bidistilled hot water. The flask was placed on a magnetic stirrer at 80 °C for 30 minutes. Cooled and quantitatively transferred into the volumetric flask on 100 cm^3. Enhanced the volume up to mark by bidistilled water [6]. The obtained solutions were carefully mixed, filtered through a membrane filter with a pore size of 0.45 µm and placed into a vial.

Using diode array detector plotted the chromatographic/spectrometric data images of the pharmacopeia standards. Standards solutions were prepared with the concentration 50-200 mg/L and performed their 5 level calibration in the manual mode. It was also applied 5 level calibration for the standard methanolic solutions of catechins, injection performed in an automatic mode with the programming of the injection volume (4, 12, 20, 28, 40 mg/L). Identification of phenolic compounds was carried out by comparison of their RT values and UV spectra with standards data. The 3D plot of chromatograms was up to 986,923, indicating the purity of identified compounds.
The calculation of the concentration was performed by the calibration method (dependence of chromatographic peak areas on mass concentration of standard sample). Based on linearity, sensitivity, precision, repeatability and accuracy the method was validated.

2.3 Total phenolic content
Determination of total phenolic content recalculating on gallic acid with absorbance maximum at 270 nm, total hydroxycinnamic acids content recalculating on rosmarinic acid at 505 nm, total flavonoids content recalculating on rutin at 410 nm in the subjected objects were conducted by spectrophotometric method of analysis – Lambda 25 Perkin Elmer UV-visible spectrophotometer (USA) with 1 cm matched quartz cells [2, 4].

3. Results and Discussion
By HPLC method of analysis it was confirmed the presence of HCA, Fl, C and T in 3 subjected species of *Veronica* genus. HPLC chromatograms of the phenolic compounds of the herbs of *Veronica prostrata* L., *Veronica chamaedrys* L., *Veronica officinalis* L. presented in the figures 1-9.

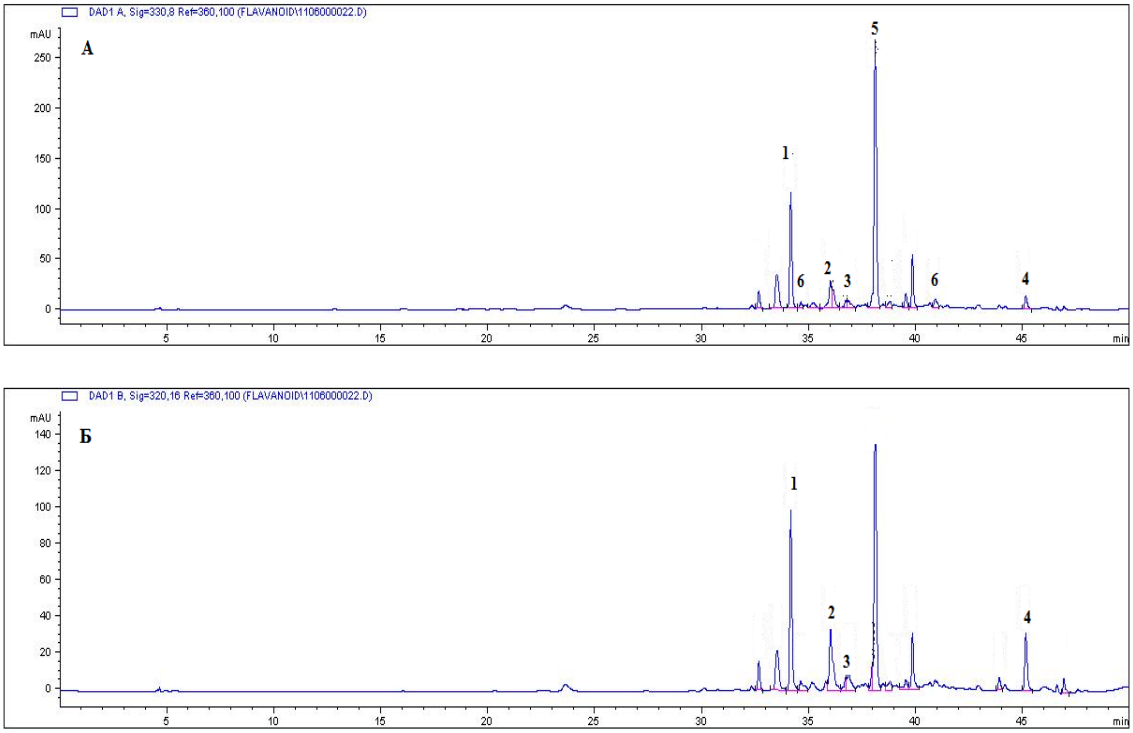


Fig 1: HPLC chromatogram of the herb of *Veronica prostrata* L. at А – λ = 330 nm; Б – λ = 320 nm: 1 — rosmarinic acid; 2 — caffeic acid; 3 — *p*-coumaric acid; 4 — ferulic acid; 5 — apigenin; 6 — apigenin glycosides

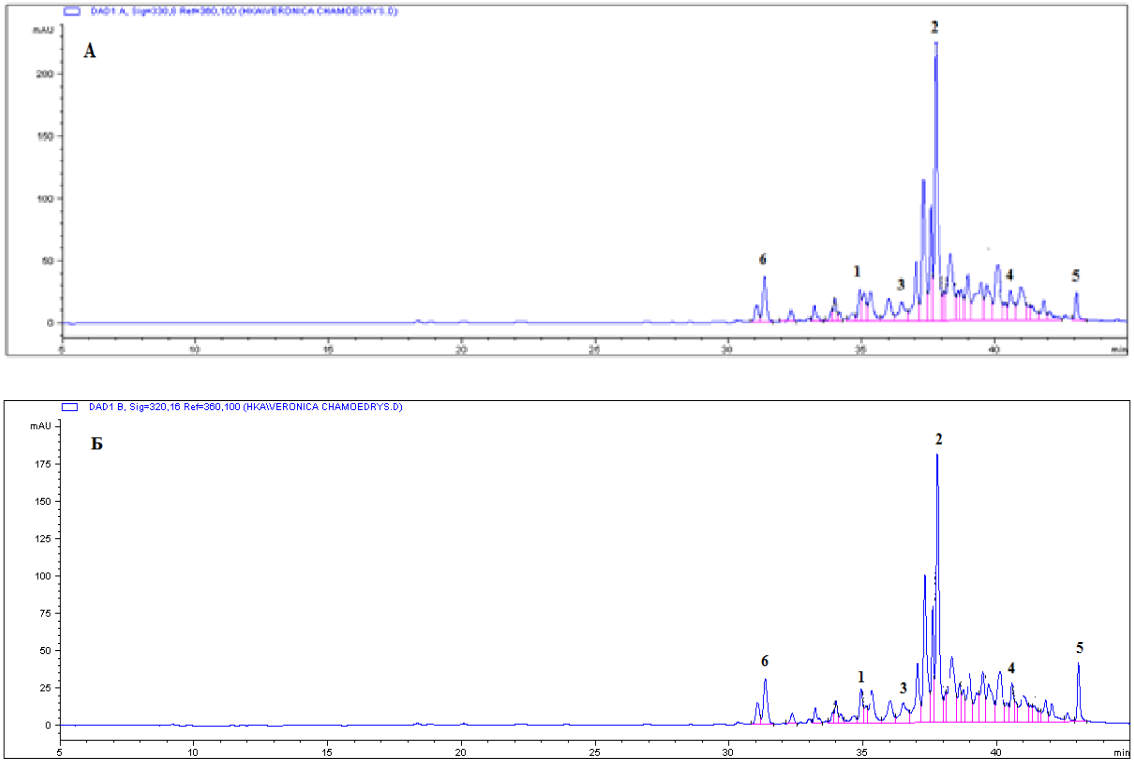


Fig 2: HPLC chromatogram of the herb of *Veronica chamaedrys* L. at А – λ = 330 nm; Б – λ = 320 nm: 1 — chlorogenic acid; 2 — rosmarinic acid; 3 — ferulic acid; 4 — caffeic acid; 5 — *p*-coumaric acid; 6 — apigenin

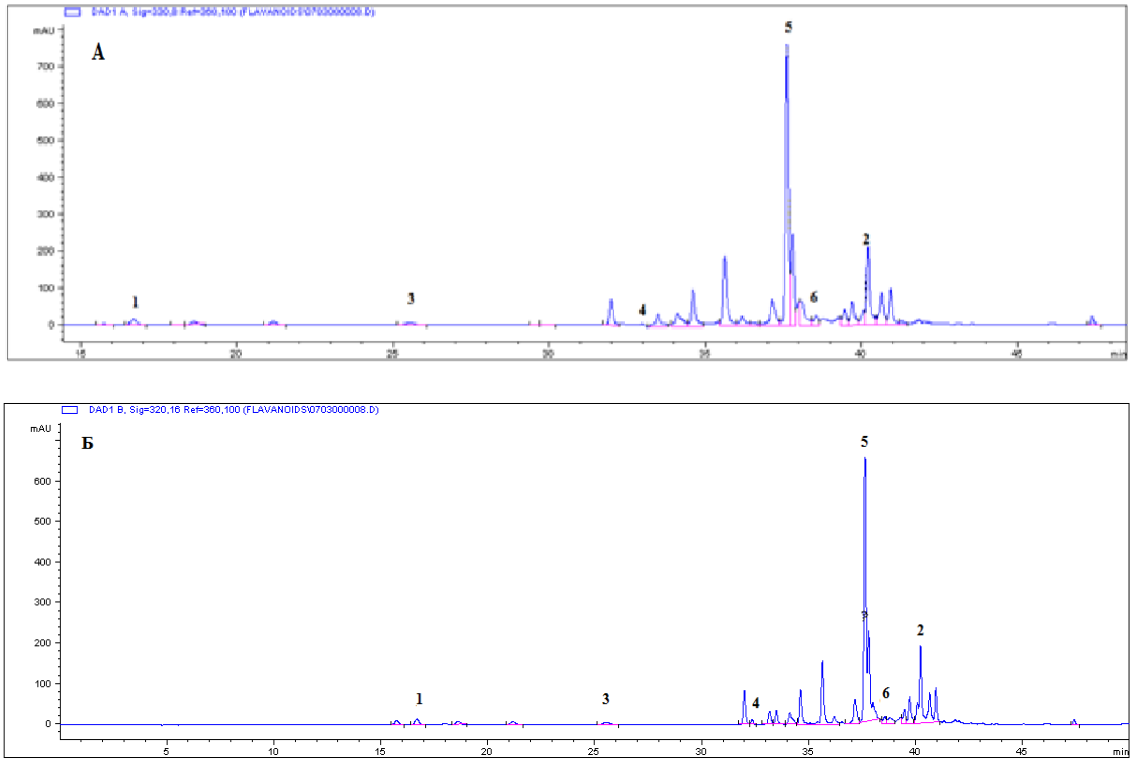


Fig 3: HPLC chromatogram of the herb of *Veronica officinalis* L. at А — λ = 330 nm; Б — λ = 320 nm: 1 — chlorogenic acid; 2 — rosmarinic acid; 3 — caffeic acid; 4 — *p*-coumaric acid; 5 — ferulic acid; 6 — apigenin

It was established the presence of rosmarinic, caffeic, *p*-coumaric and ferulic acids in the studied species of *Veronica* genus. In addition it was determined chlorogenic acid in the herb of *Veronica officinalis* L. and *Veronica chamaedrys* L. And all species of *Veronica* genus contain apigenin and its glycosides (tab. 2).

Also by HPLC analysis it was determined the quality composition and quantity content of Fl and C. in the studied species of *Veronica* genus (Fig. 4-6, tab. 2).

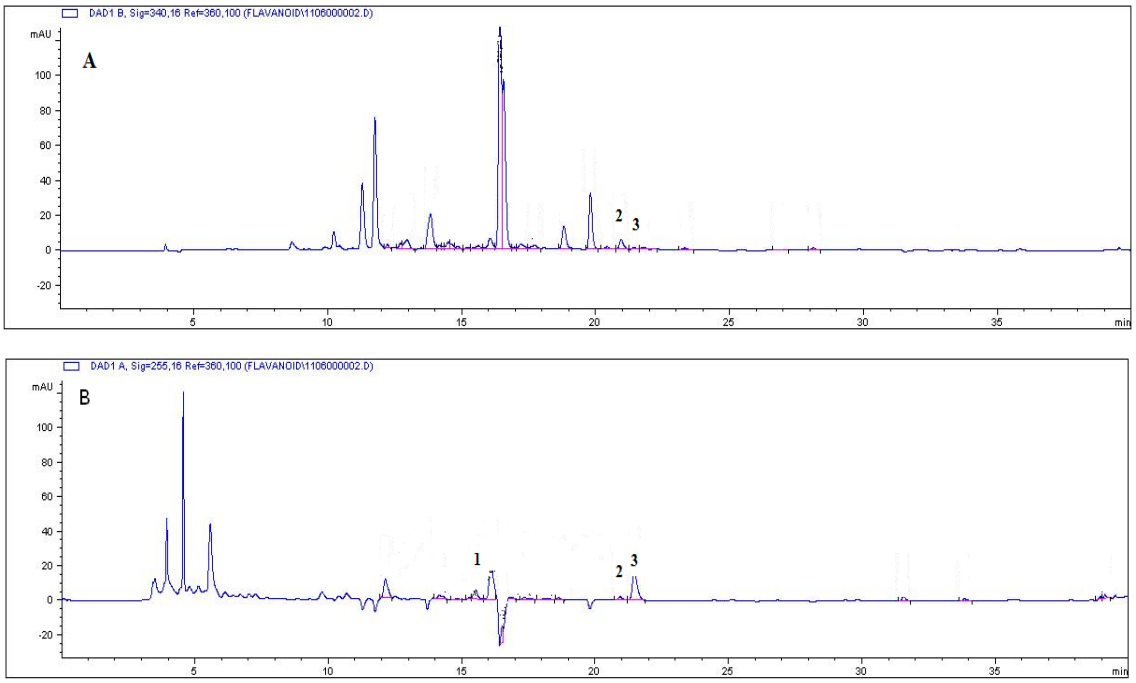


Fig 4: HPLC chromatogram of the herb of *Veronica prostrata* L.at A – λ = 340 nm; B – λ = 255 nm: 1 — hyperoside, 2 — luteolin, 3 — coumarin

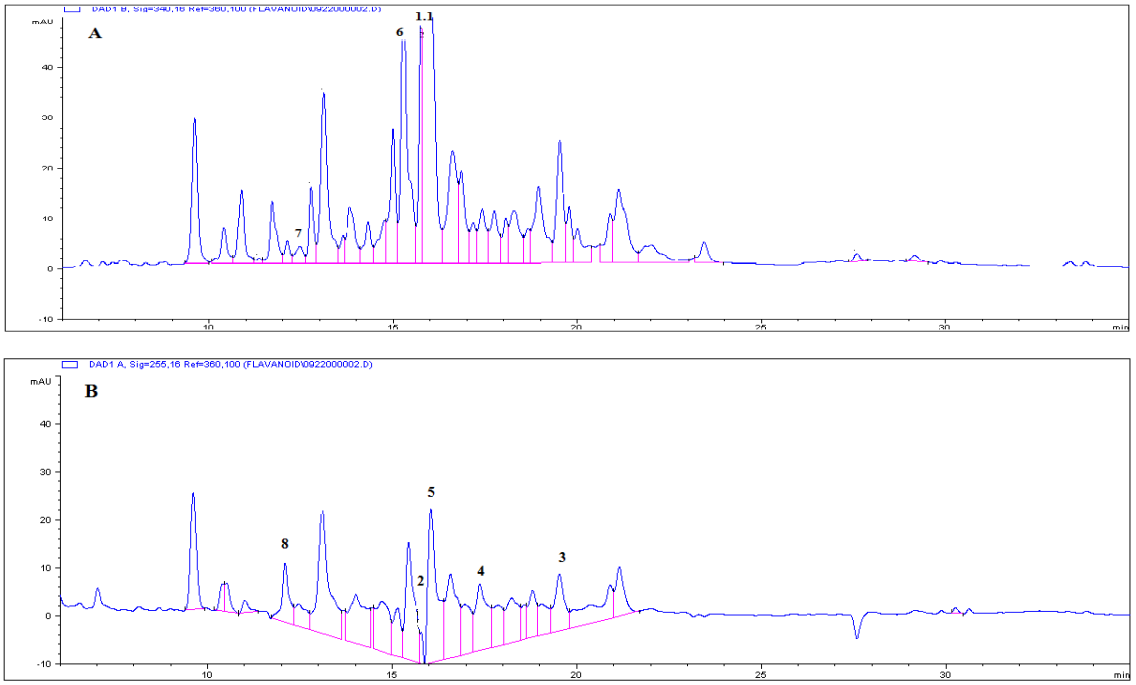
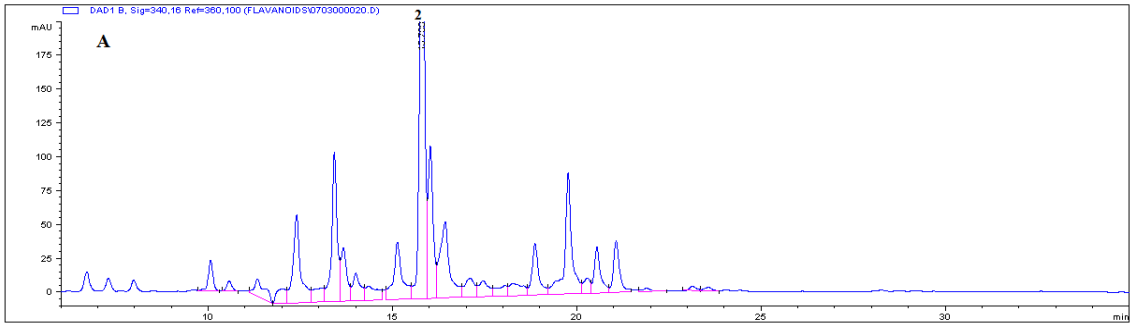


Fig 5: HPLC chromatogram of the herb of *Veronica chamaedrys* L. at A – λ = 340 nm; B – λ = 255 nm: 2 — hyperoside; 3 — luteolin; 4 — isoquercetin; 5 — rutin; 6 — umbelliferone; 7 — scopoletin; 8 — coumarin



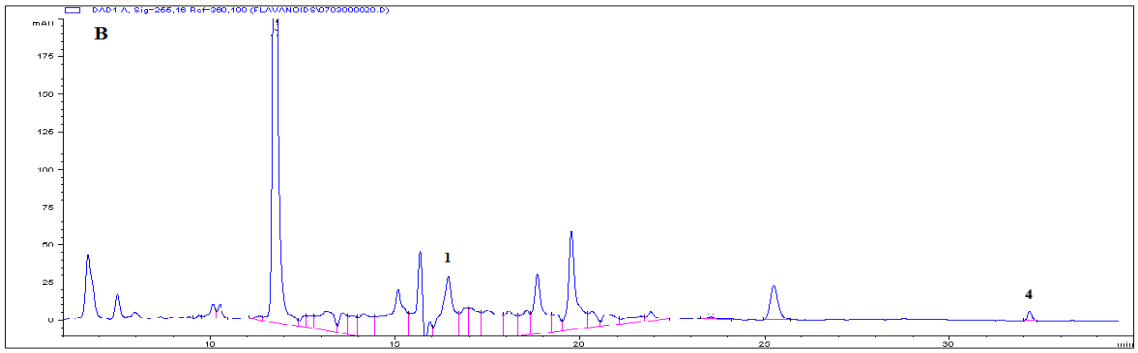


Fig 6: HPLC chromatogram of the herb of *Veronica officinalis* L. at A – λ = 340 nm; B – λ = 255 nm: 1 — isoquercetin, 2 — umbelliferone, 3 — coumarin

As a results, it was confirmed the presence of hyperoside, luteolin, isoquercetin, rutin, umbelliferone, scopoletin, coumarin in the studied species of *Veronica* genus.

By HPLC method in the subjected objects it was also determined the chromatographic profile of hydrolysable tannins: gallic and ellagic acids, gallocatechin, epigallocatechin, catechin, epicatechin, catechin gallate, epicatechin gallate (Fig. 7-9, tab. 2).

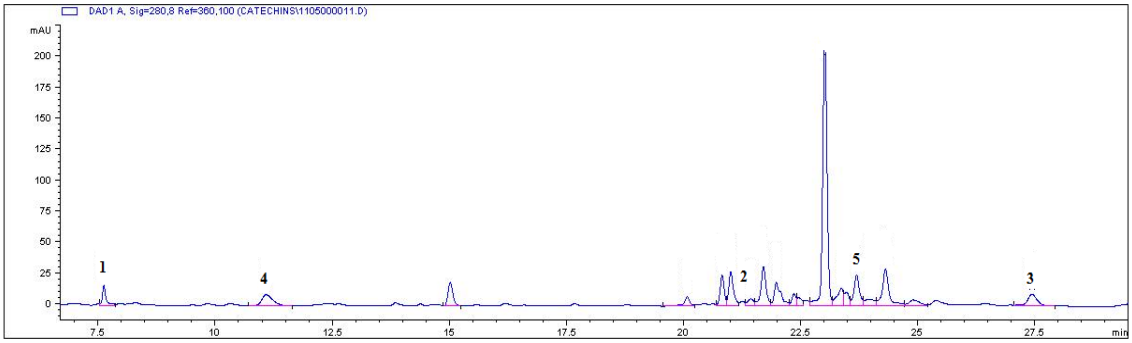


Fig 7: HPLC chromatogram of the herb of *Veronica prostrata* L.at λ = 280 nm: 1 — gallic acid; 2 — epicatechin; 3 — epicatechin gallate; 4 — ellagic acid; 5 — catechin gallate

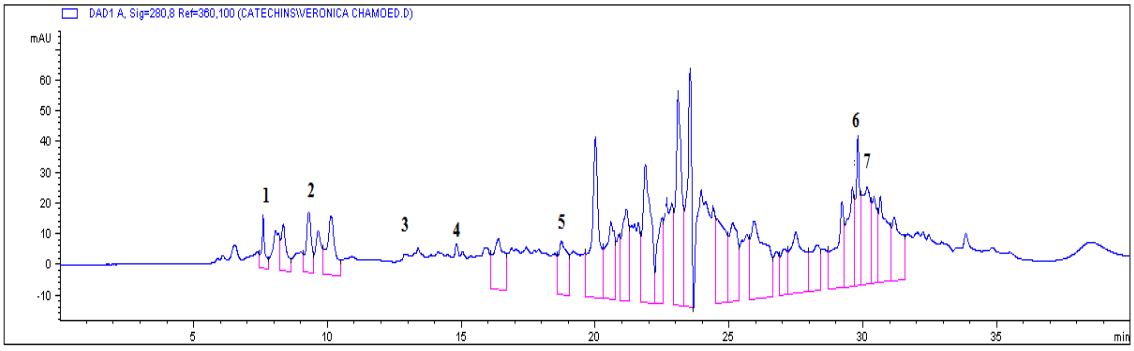


Fig 8: HPLC chromatogram of the herb of *Veronica chamaedrys* L. at λ = 280 nm: 1 — gallic acid; 2 — gallocatechin; 3 — epigallocatechin; 4 — catechin; 5 — epicatechin; 6 — catechin gallate; 7 — epicatechin gallate

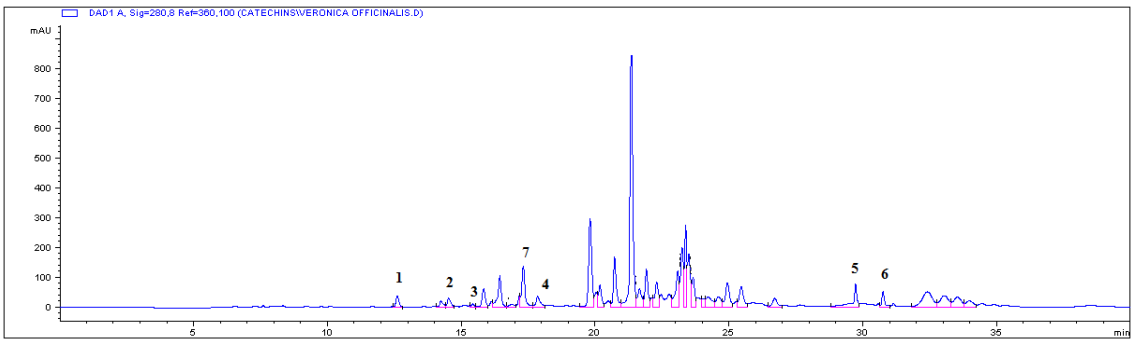


Fig 9: HPLC chromatogram of the herb of *Veronica officinalis* L. at λ = 280: 1 — gallic acid; 2 — gallocatechin; 3 — epigallocatechin; 4 — epicatechin; 5 — catechin gallate; 6 — epicatechin gallate; 7 — catechin

Table 2: Quantity content of phenolic compounds in the herbs of *Veronica prostrata* L., *V. chamaedrys* L., *V. officinalis* L. (%)

Phenolic compounds, %	*V. prostratae* L.	*V. chamaedrys* L.	*V. officinalis* L.
	Hydroxycinnamic acids		
Rosmarinic acid	0,41	1,31	1,41
Caffeic acid	0,04	0,03	0,03
p-coumaric acid	0,01	0,03	0,01
Ferulic acid	0,05	0,02	1,35
Chlorogenic acid	—	0,04	0,24
	Flavonoids		
Apigenin	0,01	0,06	0,04
Apigenin glycosides	0,33	0,01	—
Hyperoside	0,01	0,004	—
Luteolin	0,006	0,04	—
Isoquercetin	—	0,09	0,28
Rutin	—	0,26	—
	Coumarins		
Coumarin	0,07	0,04	0,02
Umbelliferone	—	0,17	0,03
Scopoletin	—	0,05	—
	Hydrolysable tannins		
Gallic acid	0,02	0,01	0,03
Gallocatechin	0,91	—	0,43
Epigallocatechin	0,34	—	0,44
—	1,45		0,07
Epicatechin	0,41	0,06	0,03
Catechin galate	0,09	0,04	0,09
Epicatechin gallate	0,05	0,03	0,30
Ellagic acid	—	0,06	—

The experimental results showed that all the studied objects contain rosmarinic, caffeic, *p*-coumaric and ferulic acids, apigenin, coumarin, gallic acid, epicatechin, catechin galate, epicatechin gallate. In addition in the herb of *V. prostratae* L. it was determined luteolin, gallocatechin, epigallocatechin, catechin, *V. chamaedrys* L. – chlorogenic acid, hyperoside, luteolin, isoquercetin, umbelliferone, rutin, scopoletine, ellagic acid, *V. officinalis* L. – chlorogenic acid, isoquercetin, umbelliferone, gallocatechin, epicatechin, catechin.

\The total content of phenolic compounds recalculating on gallic acids was (4,47 ± 0,01) % for *V. prostratae* L., (2,85± 0,005) % — *V. chamaedrys* L. and (4,36± 0,20) % — *V. officinalis* L; the total hydroxycinnamic acids content recalculating on rosmarinic was (1,66 ± 0,005) % for *V. prostratae* L., (1,74± 0,003) % — *V. chamaedrys* L. and (2,00 ± 0,007) % — *V. officinalis* L.; total flavonoids content recalculating on rutin was (2,30 ± 0,04) % for *V. prostratae* L., (3,42± 0,006) % — *V. chamaedrys* L. and (2,52 ± 0,021) % — *V. officinalis* L. (Fig. 10).

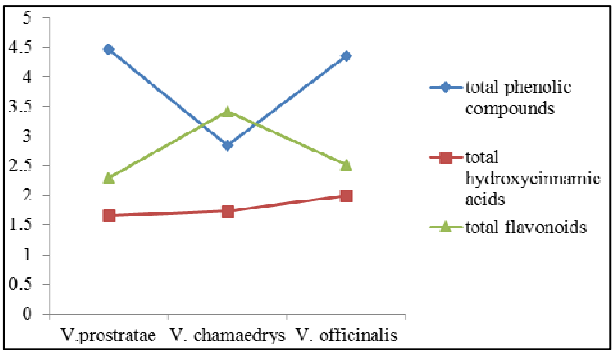


Fig 10: Total phenolic content of the herbs of subjected species of *Veronica* genus

4. Conclusions

At the first time it was studied the quality composition and quantity content of phenolic compounds in the herb of *Veronica prostrata* L., *Veronica chamaedrys* L., *Veronica officinalis* L. As a results it was established the most abundant phenolic compound for each species: *Veronica prostrata* L. – catechin (1,45%), *Veronica chamaedrys* L. – rosmarinic acid (1,31%), *Veronica officinalis* L. – rosmarinic acid (1,41%) and ferulic acid (1,35%).

Thus *Veronica officinalis* L, *Veronica chamaedrys* L., *Veronica prostrata* L., are promising sources for the development of new herbal drugs with cardioprotective, antioxidant, anti- inflammatory action.

5. Reference

1. Determination of caffeoylquinic acids and flavonoids in *Cynara scolymus* L. by high perfomance liquid chromatography / M. Häuser, M. Ganzera, G. Abel, M. Popp, H. Stuppner // Chromatographia. — 2002; 56(7/8):407-411.
2. European Pharmacopoeia, 6th edition, Strasbourg, France: Council of Europe, 2007; (2.2):2355.
3. Khoddam A, Meredith A. Techniques for Analysis of Plant Phenolic Compounds. Molecules 2013; 18:2328-2375.
4. Koshovy OM, Komisarenko AM, Kovalova AM, Malashan LM *et al.* Investigation of phenolic compounds of the leaves of Eucalyptus. Pharmacom 2005; 2-3:151-161.
5. Kozachok SS. Determination of phenolic compounds in antiallergic herbal composition by HPLC. Pharmaceutical review 2014; 4:34-40.
6. Marchishin SM, Kozachok SS. Estimation of hydroxycinnamic acids in antiallergenic tea by HPLC method. Networks scientific edition: Medicine and education in Siberia 2013, 4. http://ngmu.ru/cozo/mos/article/text_full.php?id=1101
7. Synthetic phenolic compounds obtained from the herbs and herbal extracts that are effective for the treatment and prevention of clamydia infections. Patent Ru 2246939, 2005.02.27.
8. Yakovleva GP. Pharmacognosy. Medicinal materials of the plant and animal origin, 2nd edition, St. Petersburg: Spec Lit, 2010, 863.